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The Effect of Glucocorticoids on the Activity of Monoamine Oxidase and Superoxide Dismutase in the Rat Interscapular Brown Adipose Tissue

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ABSTRACT—The effect of dexamethasone (DEX) and corticosterone (COR) on the activity of monoamine oxidase (MAO), copper-zinc superoxide dismutase (CuZn SOD) and manganese superoxide dismutase (Mn SOD) in the rat interscapular brown adipose tissue (IBAT) were studied. DEX (1 mg/kg, i.p. for two days) significantly increased MAO activity in the IBAT as compared to the corresponding controls. On the contrary, COR, in the corresponding dose (5 mg/kg), did not affect MAO activity in the IBAT. DEX also markedly enhanced the activity of both SODs in the tissue studied, while COR was ineffective. The results suggest that there exist the differences in the effect between the synthetic glucocorticoid, such as DEX, and COR, which is a natural glucocorticoid in the rat, on the activity of IBAT enzymes studied.

INTRODUCTION

The major role of brown adipose tissue (BAT) is heat production. This specialized tissue is considered to be an effector of non-shivering [10], and diet induced thermogenesis [21]. Heat production within the BAT results from the oxidation of fatty acids in mitochondria [3] without the involvement of ATP synthesis. The main regulator of the BAT activity is noradrenaline [12], which is released from the sympathetic nerve terminals, present within the tissue. The BAT metabolic activity can be modified by a number of hormones. For example, COR reduces this activity [11] whereas adrenalectomy causes its increase [14]. On the other hand, it is known that the increased oxygen consumption in the BAT may induce the generation of oxygen free radicals in mitochondria [1]. These free radicals are very toxic and hence aerobic organisms possess anti-oxidant enzymes which maintain their intracellular concentration at a low level preventing the membrane destruction. The main enzyme, which dismutates superoxide anion radicals (O_2^-) into H_2O_2 plus O_2^- is superoxide dismutase (SOD). The rat IBAT contains a relatively high content of the two main forms of SOD: CuZn SOD and Mn SOD residing in the cytosol and in the matrix of mitochondria respectively [20]. Besides, in the processes of catecholamine deamination with MAO, H_2O_2 is produced [4, 24]. Thus, the changes in the activity of some enzymes of the anti-oxidant system (SODs), along with the changes in the activity of MAO, an enzyme involved in the metabolism of noradrenaline, which is the main regulator of BAT activity, could be relevant indicators of metabolic alterations in the BAT under the hormonal influence. In order to elucidate this possibility we have studied the effect of dexamethasone and corticosterone on the activities of MAO and on two antioxidant enzymes of the superoxide dismutase family, the CuZn SOD and Mn SOD, in IBAT.

MATERIALS AND METHODS

Experiments were carried out on male rats of the Wistar strain, weighing 193–227 g at the beginning of the experiment. The animals were previously acclimated to 21±1°C, maintained under intermittent 12 hr periods of light and dark and given food and water *ad lib*. The rats were divided into 4 groups. The first group consisted of animals treated with dexamethasone (ICN, Galenika) dissolved in saline, in dose of 1 mg/kg body weight *i.p.* for 2 days. The second group of rats (control) was treated in the same way but with the saline only. The rats of the third group received corticosterone (Sigma, Chemical Co., St Louis, MO, USA) in dose of 5 mg/kg body weight, *i.p.* for 2 days. Before the injection, corticosterone was dissolved in a small amount of ethanol and diluted with saline. Parallelly, the fourth group of rats was treated with vehicle (ethanol-saline) only. On day 3 of the experiment all the animals were decapitated and their IBAT removed, placed in the appropriate medium, weighed and prepared for the measurement of the enzyme activities. Namely IBAT from each rat was minced and divided into two portions. One portion was then homogenized in cold 0.9% KCl, and used for determination of MAO activity with ^{14}C-triptamine bisuccinate as a substrate by the method of Wurtman and Axelrod [25]. The results obtained are expressed as pmol/mg of proteins/min of incubation. Total protein content was measured by the method of Lowry [18].

Another IBAT portion was homogenized at 0–4°C using 0.25 M sucrose, 0.05 M Tris and 0.1 nM EDTA adjusted to pH 7.4, with HCl. The homogenates were sonicated (at 50 W for 30 s in a Bronson model B-12 sonicator) to release the Mn SOD. The

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homogenates were then centrifuged at 6,000×g for 15 min. The supernatant was centrifuged at 85,000×g for 90 min and used for the determination of CuZn SOD and Mn SOD activities. SOD activity in the cytosol (CuZn SOD) and mitochondria (Mn SOD) was determined by the epinephrine method of Misra and Fridovich [19] and expressed as units of SOD/mg of proteins. One unit of SOD was defined as the amount of protein inhibiting the oxidation of epinephrine by 50% under the appropriate reaction conditions. Blood glucose concentration was measured with a glucose analyser (Exactech) using Dextrostix reagent strips.

IBAT mitochondria were prepared by the method of Slinde *et al.* [23] and mitochondrial protein content was estimated [18]. All results have been presented as means±SEM. Statistical significance of differences between groups was evaluated by Student's t-test.

RESULTS

Effect of dexamethasone and corticosterone on the body weight, blood glucose, total protein and mitochondrial protein content

Dexamethasone significantly lowered body weight ($P<0.005$), whereas corticosterone produced a marked body weight gain ($P<0.005$) in relation to the corresponding controls (Table. 1). At the same time, dexamethasone

TABLE 1. Effects of dexamethasone and corticosterone on body weight, IBAT weight, blood glucose, total protein and mitochondrial protein content

Treatment	Saline (Control)	Dexamethasone	Saline + Alcohol	Corticosterone
Initial body weight (g)	193.00±1.70*	221.00±3.80	227.00±3.00	224.00±5.30
Final body weight (% of initial values)	107.77	92.76	104.85	114.73
Blood glucose (nmol/l)	6.60±0.31*	6.90±1.60	6.74±2.20	7.01±1.80
IBAT weight per body weight (mg/g)	0.91	1.22	1.03	0.95
IBAT total protein content (mg/ml homog.)	5.06±0.22*	4.83±0.33	4.91±0.28	4.40±0.13
Mitochondrial protein content (mg/ml homog.)	0.30±0.02	0.26±0.03	0.29±0.01	0.17±0.01

* Mean±S.E.M. (n=6)

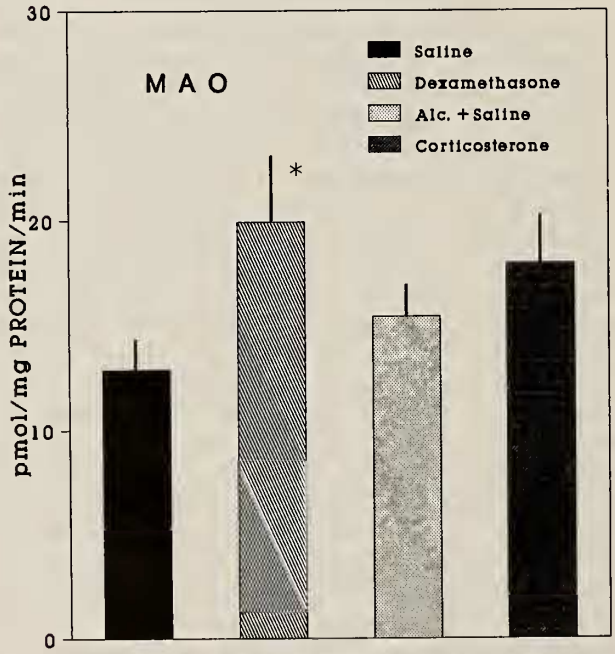

FIG. 1. The effect of dexamethasone (1 mg/kg b.w. *i.p.* for two days) or corticosterone (5 mg/kg b.w. *i.p.* for two days) on the MAO activity in the rat IBAT. Values (pmol of indol acetic acid formed per mg of protein per min of incubation) are given as means±SEM of six animals. Difference from the saline control: $^{*}P<0.05$.

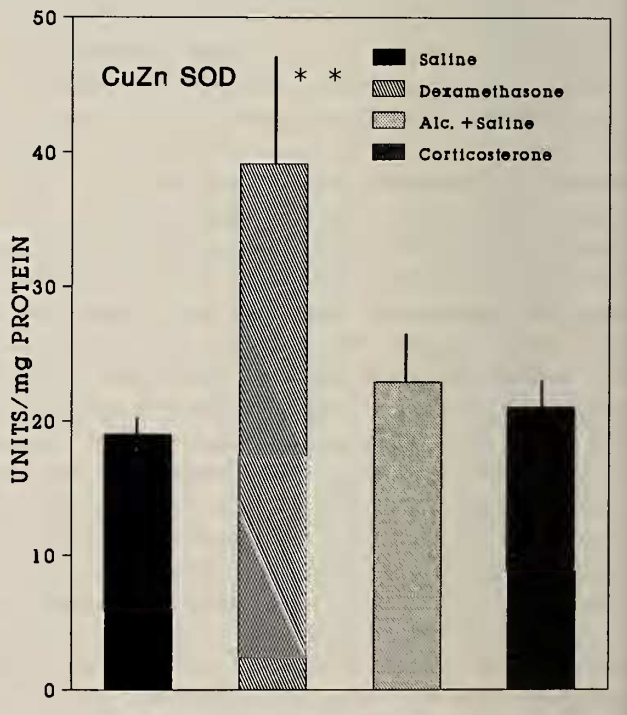

FIG. 2. The effect of dexamethasone (1 mg/kg b.m. *i.p.* for two days) or corticosterone (5 mg/kg b.m. *i.p.* for two days) on the CuZn SOD activity in the rat IBAT. Values (units per mg of protein) are given as means±SEM of six animals. Difference from the saline control: $^{**}P<0.025$.

caused a significant increase in the IBAT weight (34%, $P<0.005$), whereas corticosterone had no effect on the weight of this tissue. Both glucocorticoids showed any significant effect neither on the blood glucose level nor on the total IBAT protein content. In contrast to dexamethasone, which exerted no effect on the mitochondrial protein content, corticosterone significantly reduced it ($P<0.005$).

Effects of dexamethasone and corticosterone on the MAO activity in the rat IBAT

Dexamethasone significantly increased MAO activity in the IBAT ($P<0.05$) as compared to the saline treated controls (Fig. 1). The values for control and dexamethasone-treated animals were 14.22 ± 1.43 and 19.91 ± 3.13 pmol/mg protein/min, respectively. However, corticosterone did not markedly change the activity of this catecholamine degrading enzyme in the tissue. The respective values for control and corticosterone-treated rats were 15.42 ± 1.53 and 17.91 ± 2.30 pmol/mg protein/min.

Effects of dexamethasone and corticosterone on the CuZn SOD and Mn SOD activities in the rat IBAT

It is evident from Figure 2 that dexamethasone significantly increased CuZn SOD activity in the IBAT ($P<0.025$). The values for control and dexamethasone-treated rats were 18.98 ± 1.27 and 39.14 ± 8.40 U/mg protein respectively. Similarly, a marked enhance in Mn SOD activity in the IBAT of rats was obtained by dexamethasone treatment (Fig. 3). The respective values for the control and dexamethasone treated animals were 2.02 ± 0.30 and 4.30 ± 0.82 U/mg protein ($P<0.025$). On the other hand, corticosterone did not influence the activity of either of the SOD forms in the IBAT (Figs. 2 and 3) in respect to corresponding controls (control 22.90 ± 3.87; corticosterone-treated 21.03 ± 2.08 U/mg protein, for CuZn SOD and control 3.43 ± 0.85; corticosterone treated 4.43 ± 0.18 U/mg protein, for Mn SOD).

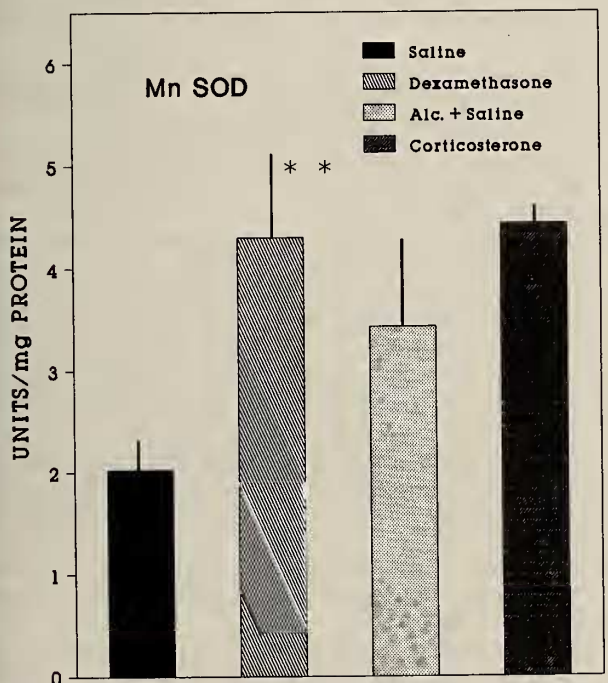

FIG. 3. The effect of dexamethasone (1 mg/kg b.m. *i.p.* for two days) or corticosterone (5 mg b.m. *i.p.* for two days) on the Mn SOD activity in the rat IBAT. Values (units per mg of protein) are given as means ± SEM of six animals. Differences from the saline control: $^{**}P<0.025$.

DISCUSSION

In the present study we observed that dexamethasone significantly lowered body weight, which is in accordance with the results of Fleck *et al.* [9]. This is probably the consequence of the stimulating effect of this hormone on the IBAT metabolic activity which leads to the increased energy expenditure [16]. However, corticosterone produced the significant body weight gain, which probably resulted from its inhibitory action on the secretion of corticotrophin releasing hormone (CRH). Namely, it is known that CRH enhances the sympathetic activity in the IBAT and increases energy expenditure [2, 7]. Therefore, it is possible to suppose that exogenously applied corticosterone inhibits CRH secretion and consequently, the sympathetic nervous system activity in the IBAT as well as the energy expenditure, which results in the body weight gain. We have also shown that dexamethasone markedly increased the IBAT weight while corticosterone had no significant effect. This increase in the IBAT weight, which is not accompanied by the change in the IBAT total protein content, may result from the possible intense lipogenesis, induced by insulin, the release of which is stimulated by dexamethasone [13].

Our results also clearly indicate the different actions of two glucocorticoids, dexamethasone and corticosterone, on the activity of catecholamine degrading enzyme MAO and the main enzymes for antioxidant defence, CuZn SOD and Mn SOD, in the IBAT of rats. Namely, dexamethasone, a highly potent synthetic glucocorticoid, the half life of which in the circulation is markedly longer than that of corticosterone, increased the activity of all IBAT enzymes studied. At the same time, short-term treatment of rats with corticosterone, which is a natural glucocorticoid in the rats, did not change markedly either the activity of catecholamine degrading enzyme MAO or the activity of any of the two SOD forms. However, the mechanism of action by which glucocorticoids alter MAO and SODs activities, *in vivo*, is still unknown. It is not known whether dexamethasone and corticosterone exert the direct effect on the activity of all enzymes studied, or their influence is mediated by changes in the CRH production and consequently in the activity of IBAT innervating sympathetic nerves. In fact our results concerning the stimulating effect of dexamethasone on the activity of the main enzymes for antioxidant defence and on catecholamine degrading enzyme MAO in the IBAT are apparently not in agreement with general idea that corticoids have a depressing effect on IBAT activity and thermogenin content in rodents

[15, 22]. However, having in mind the observations that the prolonged dexamethasone treatment can increase thermogenin content and thermogenin mRNA expression [16], it is possible to suppose that this glucocorticoid, acting directly on the IBAT, increases both metabolic and MAO activities. Therefore, under the present experimental conditions the production of free radicals might be enhanced and consequently the induction of enzymes for antioxidant defence.

Unlike dexamethasone, corticosterone did not change significantly either the activity of MAO or that of either of the two SOD forms. The failure of corticosterone to affect these activities might have been predicted if we bear in mind the following evidence: first, corticosterone is one "antibrown fat" hormone [11, 15] which inhibits the metabolic activity of BAT [26]; second, corticosterone reduces in rats both the integrated sympathetic nervous system activity and direct sympathetic activity within the IBAT, as judged by the decreased urinary noradrenaline excretion and noradrenaline turnover rate in the IBAT [5], and finally, it is well known that changes in the circulating corticosterone level induce reciprocal effects in hypothalmic CRH production [26]. Namely, York [26] showed that CRH, given centrally, increases the firing rate of sympathetic innervation of IBAT in both, lean and obese rats. Le Feuvre *et al.* [17] also observed that central administration of CRH produces an activation of the brown adipose tissue thermogenesis in the rats. Since CRH enhances sympathetic activity, it is possible to suppose that exogenously applied corticosterone inhibits the production of CRH, by a negative feed-back mechanism, decreasing in this way both the sympathetic nervous system activity and noradrenaline turnover in the IBAT [5]. Therefore, under these experimental conditions, the production of free radicals and consequently the increase in the activity of SODs were not possible. Bearing in mind the data mentioned above, it may be concluded that the differences in the effect between dexamethasone and corticosterone on the activity of IBAT enzymes studied result from the different pathways of their action. It seems that dexamethasone acts directly on the IBAT through glucocorticoid (GR) receptors, the existence of which was proved by Feldman [8]. These receptors have a few times higher affinity for dexamethasone than for corticosterone [6]. However, corticosterone probably acts indirectly through the changes in the activity of efferent sympathetic nerves, which innervate this specialized tissue.

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